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Modified Hodge Test versus Indirect Carbapenemase Test: Prospective Evaluation of a Phenotypic Assay for Detection of *Klebsiella pneumoniae* Carbapenemase (KPC) in *Enterobacteriaceae*

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The currently recommended phenotypic test for the detection of carbapenemase-producing members of the family *Enterobacteriaceae* is the modified Hodge test (MHT). However, the MHT lacks specificity. Here we demonstrate an alternative phenotypic test, the indirect carbapenemase test, for the detection of bla_{KPC}-producing isolates that has specificity superior to that of the MHT for non-*Klebsiella Enterobacteriaceae*.

The last decade has witnessed the dramatic emergence and worldwide dissemination of carbapenem resistance in *Enterobacteriaceae*. In the United States, the most common mechanism of carbapenem resistance is the production of the Ambler class A serine β-lactamase *Klebsiella pneumoniae* carbapenemase (KPC) (1). The bla_{KPC} gene product has the ability to hydrolyze all penicillins, cephalosporins, monobactams, and carbapenem antibiotics, which often leaves clinicians with few therapeutic options. As a consequence, infections due to carbapenemase-producing *Enterobacteriaceae* (CPE) carry a high mortality rate, reaching 40% or higher in some studies (2–5).

The bla_{KPC} gene is carried on resistance plasmids that are readily transmitted between bacterial strains and species, making the prevention of in-hospital transmission especially difficult (6–8). Accurate recognition of CPE in the clinical microbiology laboratory is central to controlling the spread of these organisms in the health care setting (9). However, identification of CPE from clinical isolates can be challenging. Molecular detection of a specific carbapenemase gene is the gold standard, but this method is impractical for many clinical laboratories.

In 2009, the Clinical and Laboratory Standards Institute (CLSI) suggested phenotypic evaluation for the presence of a carbapenemase in *Enterobacteriaceae* with elevated MICs of one or more carbapenems by using the modified Hodge test (MHT) (9). The MHT has been found to be a useful tool for the detection of CPE but lacks specificity for serine carbapenemases (10–13). We previously reported a heterogeneous outbreak of KPC-producing *Enterobacteriaceae* (7, 14). Starting in late 2009, there was an increase in *Enterobacteriaceae* isolates (mostly *Enterobacter* spp.) that were MHT positive but bla_{KPC} PCR negative. This prompted us to prospectively evaluate phenotypic screening approaches for carbapenemase production. Here we compare the MHT and the indirect carbapenemase test (ICT), originally described by Moland et al. (15). Those investigators also described a direct carbapenemase test with imipenem, but we did not use it because some of our isolates expressed such high levels of resistance that no evaluable phenotypic result was possible.

This study was conducted at the University of Virginia Medical Center, a 619-bed tertiary-care hospital in central Virginia, from 1 May 2010 to 31 December 2011. One hundred twenty-seven isolates of *Enterobacteriaceae* with ertapenem MICs of ≥1 μg/ml were prospectively collected during the study period. Given the heterogeneous nature of our earlier outbreak (7), all species of *Enterobacteriaceae* were examined. Isolates were collected from urine ($n = 67$), respiratory ($n = 17$), blood ($n = 12$), abdominal ($n = 9$), wound ($n = 7$), and other ($n = 15$) specimens. Isolates were identified and their antibiotic susceptibility profiles were determined by using the VITEK2 system with the GN ID card and the AST-GN45 card (bioMérieux, Durham, NC), respectively. The study population consisted of *Enterobacter* sp. ($n = 74$), *Klebsiella* sp. ($n = 38$), *Citrobacter freundii* ($n = 8$), and *Escherichia coli* ($n = 7$) isolates. All isolates were evaluated for carbapenemase production by the MHT and an ICT and for the presence of bla_{KPC} by PCR (14).

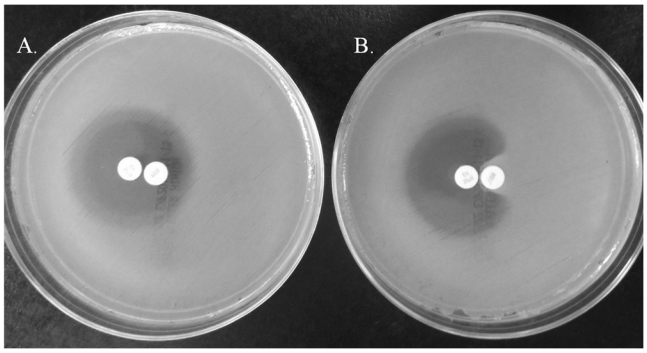

FIG 1 ICT with imipenem. Two or three colonies of the test isolate are under each EDTA disk. (A) Negative test without invagination of carbapenem-susceptible *E. coli* ATCC 25922 growth. (B) Positive test with invagination of the lawn.

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TABLE 1 Correlation of phenotypic test results with *K. pneumoniae* carbapenemase (bla_{KPC}) PCR results organized by species[a]

Test result	All isolates ($n = 127$)		*Klebsiella* spp. ($n = 38$)		*Enterobacter* spp. ($n = 74$)		*C. freundii* ($n = 8$)		*E. coli* ($n = 7$)	
	bla_{KPC} PCR positive ($n = 56$)	bla_{KPC} PCR negative ($n = 71$)	bla_{KPC} PCR positive ($n = 32$)	bla_{KPC} PCR negative ($n = 6$)	bla_{KPC} PCR positive ($n = 18$)	bla_{KPC} PCR negative ($n = 56$)	bla_{KPC} PCR positive ($n = 6$)	bla_{KPC} PCR negative ($n = 2$)	bla_{KPC} PCR positive ($n = 0$)	bla_{KPC} PCR negative ($n = 7$)
MHT positive	52	26	29	0	17	21	6	2	0	3
MHT negative	4	45	3	6	1	35	0	0	0	4
ICT positive	51	3	27	0	18	2	6	1	0	0
ICT negative	5	68	5	6	0	54	0	1	0	7

[a] All data are numbers of isolates.

The MHT was performed as previously described, with a 10-μg imipenem disk (16). The ICT method uses an EDTA disk. EDTA is used here not as a cation-chelating agent to inhibit metallo-β-lactamases but rather to lyse bacterial cells and release the carbapenemase. For this test, a suspension of a carbapenem-susceptible *E. coli* strain (ATCC 25922; American Type Culture Collection, Manassas, VA) was prepared in saline equal to a McFarland 0.5 turbidity standard and inoculated onto Mueller-Hinton agar (Remel, Lenexa, KS) to achieve a confluent lawn. Two or three colonies of the test isolate were applied to a 0.1 mM EDTA disk (Becton, Dickinson, Sparks, MD) by touching the tops of a few well-isolated colonies to coat the EDTA disk. The inoculated EDTA disk was placed with the bacterial inoculum (organism) side down on the lawn abutting a 10-μg imipenem disk (Becton, Dickinson). The plate was then incubated overnight at 35°C in ambient air. Indentation of growth toward the imipenem disk indicated a positive test for a serine carbapenemase (Fig. 1). Weekly quality controls for the ICT were *K. pneumoniae* ATCC strains 1705 (MHT and bla_{KPC} positive) and 1706 (MHT and bla_{KPC} negative).

To detect bla_{KPC} and other carbapenemases, DNA was obtained by suspending one or two colonies of each test isolate in 100 μl of nuclease-free water and heating it at 95°C for 10 min. Samples were spun at 10,000 rpm for 1 min. The resulting supernatant was used as the bacterial DNA template for PCR. PCR amplification of bla_{KPC} was performed as previously described (14). All isolates that had a positive phenotypic test and were PCR negative for bla_{KPC} also underwent PCR assays for other carbapenemases (bla_{VIM}, $bla_{OXA\text{-}48\text{-}like}$, and alternative primers for bla_{KPC}) using previously described primers (17) and for bla_{NDM}-directed PCR (5′-3′ forward, GGTTTGGCGATCTGGTTTTC; 5′-3′ reverse, CGGAATGGCTCATCACGATC) under the same conditions used for the KPC PCR. All reaction mixtures had positive controls. A positive bla_{KPC} PCR result was used as the true positive in the evaluation of MHT and ICT sensitivity and specificity. The presence of bla_{IPM} was not tested because of the lack of a positive control and because bla_{IPM} has been described only once for *Enterobacteriaceae* (*K. pneumoniae*) in the United States (18).

In total, 44% (56/127) of the isolates evaluated (i.e., with ertapenem MICs of ≥1 μg/ml) were found to be bla_{KPC} positive. The presence of bla_{KPC} varied by species; 24% (18/74) of the *Enterobacter* sp. isolates, 75% (6/8) of the *C. freundii* isolates, and 84% (32/38) of the *Klebsiella* sp. isolates were bla_{KPC} positive. Interestingly, all seven *E. coli* isolates with elevated ertapenem MICs identified during the study period were bla_{KPC} negative.

bla_{KPC}-positive isolates generally had higher ertapenem and imipenem MICs (correlation coefficients, 0.61 and 0.68, respectively) than bla_{KPC}-negative isolates. Cefepime MICs did not have the same association with KPC (correlation coefficient, 0.21). Fifty-five percent of the bla_{KPC}-producing isolates were considered cefepime susceptible (16). The median MICs of ertapenem, imipenem, and cefepime for the bla_{KPC}-negative and bla_{KPC}-positive isolates were 2, ≤1, and ≤1 μg/ml and ≥8, 4, and 8 μg/ml, respectively.

The results, broken down by bla_{KPC} PCR results, are shown in Table 1. The overall sensitivity of the ICT was 90.0%, and that of the MHT was 92.7% (Table 2). The lack of sensitivity of the ICT was seen entirely in the *K. pneumoniae* population, where the ICT had a sensitivity of 83.9%. The overall specificity of the ICT was 95.8%, and that of the MHT was 63.4%. Both the ICT and the MHT had false positives with *Enterobacter* spp. and *C. freundii*, but only the MHT gave false-positive results with *E. coli*. Positive MHT and negative KPC PCR results were obtained with all four *Enterobacter* species tested (*Enterobacter cloacae*, *E. aerogenes*, *E. amnigenus*, and *E. asburiae*).

TABLE 2 Performance characteristics of the MHT versus those of the ICT

Parameter	All species ($n = 127$)		*Enterobacter* spp. ($n = 74$)		*Klebsiella* spp. ($n = 38$)		*C. freundii* ($n = 8$)		*E. coli* ($n = 7$)	
	ICT	MHT	ICT	MHT	ICT	MHT	ICT	MHT	ICT	MHT
Sensitivity (%)	90.0	92.7	100	94.4	84.3	90.3	100	100	NA[a]	NA
Specificity (%)	95.8	63.4	96.4	62.5	100	100	50.0	0	100	57.1
Positive predictive value (%)	94.4	66.7	89.5	44.7	100	100	85.7	75.0	NA	0
Negative predictive value (%)	93.1	91.8	100	97.2	54.5	66.7	100	NA	100	100
Likelihood ratio positive	22.5	2.53	27.8	2.51	Infinite	Infinite	2	1		
Likelihood ratio negative	0.10	0.12	0	0.09	0.16	0.1	0	0		

[a] NA, not applicable.

The negative likelihood ratios of the MHT and the ICT are roughly equivalent (Table 2). However, the positive likelihood ratio of the ICT is >10-fold higher than that of the MHT and 2-fold higher for *Enterobacter* spp. and *C. freundii*, respectively.

Among the 26 bla_{KPC}-negative isolates with positive phenotypic test results (3 ICT positive, 26 MHT positive, and 2 both ICT and MHT positive), $bla_{VIM-1,2}$, bla_{OXA-48}, and bla_{NDM} carbapenemases were not detected by molecular analysis.

Accurate determination of whether an isolate produces a carbapenemase can inform treatment, as several studies have shown the superiority of combination therapy when KPC is present even when the MICs remain low (4, 5, 19). Noteworthy in this study is the finding that five of the bla_{KPC}-positive isolates had imipenem MICs of ≤1 μg/ml. We also found 26 MHT-positive, bla_{KPC}-negative isolates. It is not clear that these isolates pose the same infection control risk as KPC-producing *Enterobacteriaceae* (2). The sensitivity for the identification of KPC-producing *K. pneumoniae* was lower with the ICT than with the MHT. Therefore, the ICT may not be the ideal screening test for the detection of KPC production among *K. pneumoniae* isolates.

The present study suggests that the MHT advocated by the CDC and the CLSI has specificity inferior to that of the ICT for the detection of carbapenemase production by KPC-producing non-*Klebsiella Enterobacteriaceae*. The ICT can assist clinical laboratories in accurately identifying *Enterobacter* sp., *C. freundii*, and *E. coli* isolates that do not harbor bla_{KPC} yet have an elevated ertapenem MIC.

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